THE PERMEABILITY OF FROG SKIN TO UREA.

II. The Effect of Dextrose and Sucrose.

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Introduction.

Loeb (1) in a series of papers on the passage of potassium through the membranes of *Fundulus* eggs demonstrated that this substance would pass through in the presence of other electrolytes, but failed to do so with various non-electrolytes. The explanation that he offered and termed the "salt effect" could apply only to electrolytes.

Since the experiments of Loeb on potassium, there has been a good deal of other work comparing the permeability of tissues to electrolytes in the presence of other electrolytes and in the presence of non-electrolytes. Hoeber and Memmesheimer (2) showed that basic dyes enter red blood corpuscles if dissolved in salt solutions, but not if they are dissolved in sugar or glycocoll solution. Wertheimer (3) confirmed the need of electrolytes for the penetration of basic dyes through frog skin. Hiruma (4) reported that blood corpuscles take up less ammonium and alkaloid salts from a sugar solution than from a salt solution, while more salicylate or thiocyanate is taken up from the sugar solution. With frog muscle he found that basic dyes, alkaloid salts, salicylate, and thiocyanate have their penetration favored by sucrose as have also iodide and acid. The sugar checked only sodium hydroxide.

Embden and Adler (5) noted that if frog muscles were immersed in isotonic sugar solution, phosphoric acid would pass out, and the muscles would lose their contractility, although even after a stay of several hours in the sugar solution they would recover upon being again placed in Ringer's solution. Phosphoric acid was also given off in Ringer's solution if the muscles were fatigued or injured. The authors believed there

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was a direct connection between the permeability for phosphoric acid and a non-functional, *i.e.*, an injured, condition of the muscle.

Among the papers describing the conditions for the permeability of non-electrolytes, probably no other is so free from the objection that the permeability reported might be due to injury as an article by Reid (6) in which he found that absorption of glucose from the intestine is favored by the presence of salts. Hiruma (4) in the paper referred to above reported that the permeability of blood corpuscles for urea is not decreased but probably increased by the presence of other non-electrolytes.

Wertheimer (3) claimed that if frog skin had its inner surface in contact with solutions of non-electrolytes more glucose would penetrate than was the case when Ringer's solution was employed in the same way. In an earlier paper (7) he had reported that sugar passed through the frog skin into Ringer's solution if the sugar was in contact with the inner surface of the skin and the Ringer's solution on the outer surface, while no sugar passed if the direction of the skin was reversed. The durations of the experiments were fifteen hours or more. Bauer (8) objected that Wertheimer secured more permeability to sugar under the conditions that are more injurious to the skin; that is, with sugar in contact with the inner surface of the skin. In his last paper Wertheimer (9) has again stated that if a non-electrolyte bathes the inner surface, frog skin is permeable to sugar; if an electrolyte bathes the inner surface, it is impermeable. This is just the condition that Bauer maintains is most injurious, so some evidence as to the state of the skins is needed.

Most of the writers mentioned confirm the influence of electrolytes in favoring the permeability of tissues to other electrolytes. The evidence in regard to their effect on the permeability of tissues to non-electrolytes is both more meagre and more conflicting. Since the permeability of frog skin to urea has been shown in the preceding paper (10) to be decidedly influenced by the kind of salts in contact with the tissues, the writer wished to see what effect the absence of electrolytes, or the presence of another non-electrolyte would have on the penetration of the non-electrolyte, urea.

The permeability of frog skin to urea as influenced by electrolytes and by non-electrolytes may readily be ascertained, since direct chemical determinations of the amount of the substance passing through the skin may be made. Urea has the further advantage of penetrating so readily that the tissues may maintain their vitality through the period of experimentation. Hence it seemed of interest to find how much urea will pass through frog skin when it is bathed in Ringer's solution and when it is bathed in sugar solutions.

In experiments carried out during the winter of 1922–23 much less urea penetrated into sucrose solutions than into Ringer's solution. As soon as facilities for the work could be secured again, the experiments were repeated and extended to dextrose. While the results quoted are in most cases the more recent ones, they do not differ in kind from those obtained several years previously.

Materials and Methods.

The procedure was similar to that described in the foregoing paper (10). Matched pairs of frog skin were put over 16 mm. tubes. Into one tube of each pair was measured a convenient amount, usually 2.5 cc. of the sugar solution; into the other, the same amount of Ringer's solution. Each tube was then placed with its other surface bathed by the urea solution. All solutions referred to were isotonic except the urea solution. which was five per cent. In preparation for the experiment, each skin was usually washed about an hour in the particular solution that was to be used on the inside of the tube. The exposure to urea was continued for varying times. Series of experiments were carried out with both dextrose and sucrose.

When the study of the permeability of frog skin to urea was begun, much time was spent in trying to discover if the orientation of the skin made any difference in its permeability to urea. Przylecki (11) has reported that urea penetrates frog skin equally well in both directions. Under the conditions employed in this paper it was found to penetrate readily in either direction, while no marked difference in the rate of penetration in the two directions was shown except in tubes where a solution used might be more injurious to one side of the skin than to the other. For example, if sugar solution was in contact with the inner surface of the skin, after five or six hours urea penetrated much more rapidly from the outer surface than was the case if the reverse relations held. For this reason, while results are reported below for both directions, it is considered that the more significant ones were obtained with skins that had their inner surfaces in contact with urea solution, which seems fairly harmless for the tissue, and the outer surface in contact with the sugar solution. The use of solutions abnormal for the skins is discussed in detail in the recent article by Bauer (8).

Data.

Throughout the tables "normal" direction indicates that the inner surface of the skin was toward the tube and hence in contact with the sugar or Ringer's solution respectively, and the outer surface in contact with the urea; while "turned" indicates the reverse relation. The amount of urea penetrating was determined by the Van Slyke-Cullen method, and is expressed in milligrams in all tables. The figures for matched skins are given in parallel columns.

The data outlined in Table I. show the kind of results always obtained when pure sugar solution and Ringer's solution were compared with the skins turned in the two directions. The skins were washed forty minutes in Ringer's or dextrose solution, respectively. Penetration was allowed to continue for three hours and forty minutes.

Table I.

4/2/23.

	Isotonic Dextrose Solution.	Ringer's Solution.
	Skins "Normal" Direction.	
	Mg. Urea.	Mg. Urea.
	5.88	9.72
	4.50	9.00
	5.40	10.20
Average	5.26	9.84
	Skins "Turned."	
	2.16	14.46
	2.46	15.60
	2.40	17.52
Average	2.34	15.86

In Tables II. and III. are presented typical data showing the penetration of urea dissolved in varying concentrations of dextrose. The Ringer's and dextrose solutions were in contact with the inner surfaces. The percentage of isotonic sugar solution is indicated in the first column; it is made up to 100 cc. with Ringer's solution in each case. The skins were washed for one hour in the respective solutions. Penetration was allowed to continue for three hours. The skins were "turned."

Table II.

11/30/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	7.32	7.86
75	5.16	7.44
85	5.22	8.34
90	7.50	7.86
95	1.68	9.48
100	0.96	8.04

Table III.

12/1/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	3.72	5.88
75	2.76	7.74
85	4.08	6.66
90	4.80	7.02
95	2.64	8.70
100	1.20	4.74

Results with the skins in the reverse relation are given in Tables IV. and V. The skins had the inner surface in contact with the Ringer's or sugar solutions, and the outer side in contact with five per cent. urea. The skins were washed for one hour in the respective solutions. Penetration continued for three hours, and the skins were in the "normal" direction.

The results with sucrose differed no more from those obtained with dextrose than did results with either sugar alone when different skins were employed, as is indicated by the data recorded in Tables VI. and VII. The skins were "turned" and washed for one hour in the respective solutions. The time of penetration was three hours in all cases.

Table IV.

12/3/25.

Per Cent. Dextrose.	Isotonic Dextrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	5.10	5.28
75	4.32	7.80
85	2.76	4.86
90	2.52	3.96
95	4.20	5.64
100	2.34	5.76

Table V.

12/4/25.

Per Cent. Dextrose.	Isotonic Dextrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	7.08	6.12
75	4.50	8.10
85	6.12	6.48
90	6.88	6.90
95	5.82	7.80
100	4.44	7.74

Table VI.

3/2/26.

Per Cent. Sucrose.	Isotonic Sucrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	7.26	9.72
75	8.46	9.06
85	6.00	8.58
90	2.34	9.06
95	3.96	8.34
100	0.30	8.22

Table VII.

3/1/26.

Per Cent. Sucrose.	Isotonic Sucrose + Ringer's Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
50	6.24	6.84
75	9.12	10.50
85	7.02	8.58
90	5.70	9.18
95	4.98	10.68
100	0.54	8.76

Reference has been made to the greater permeability to urea in sugar solutions if the exposure is prolonged beyond three or four hours. Table VIII. contains typical results showing more penetration of urea in the solution of pure dextrose than in the

Table VIII.

2/27/25.

Isotonic Dextrose + Ringer's Solution.		Ringer's Solution.
Per Cent. Dextrose.	Mg. Urea.	Mg. Urea.
50	4.14	6.60
75	3.30	9.72
85	4.80	7.98
90	3.18	3.18
95	4.20	4.98
100	10.32	6.66

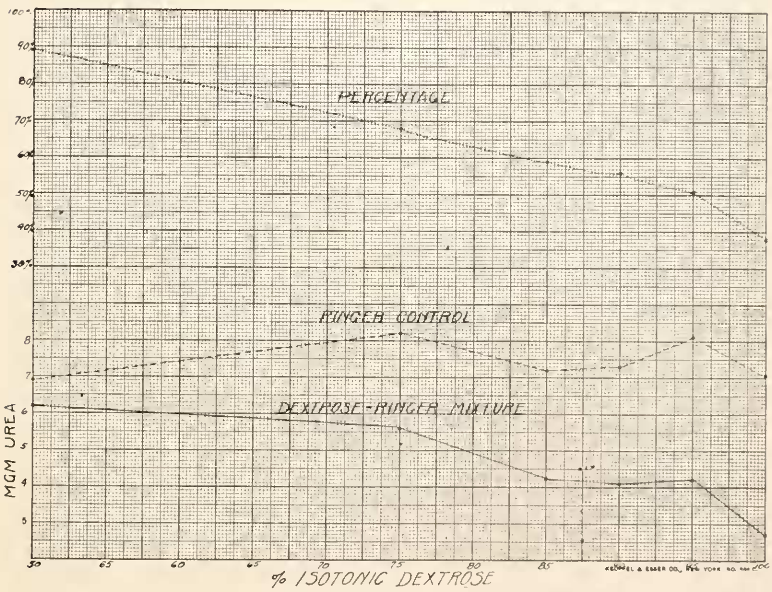

Chart I. The chart represents the composite results obtained in ten experiments.

The solid line represents the amount of urea in milligrams penetrating the skins in the Dextrose-Ringer's mixtures.

The broken line represents the amount of urea in milligrams penetrating the skins in the control experiments when Ringer's solution alone was used.

The dotted line represents the percentage of permeability on the basis of the permeability with Ringer's solution alone, which is taken to be 100 per cent.

control of Ringer's solution; and less penetration than into the controls in solutions containing dextrose with sufficient amounts of Ringer's to protect the skin against too rapid death. The skins were in the "normal" direction; washed for one hour and forty-five minutes; penetration five hours.

In order to compare the penetration of urea through dead frog skin with that through living skin, the skins were killed in several ways: by exposing to 60 per cent. ethyl alcohol; to 5 per cent. formalin; or to 0.5 per cent. sodium fluoride, from forty-five minutes to one hour. Often skin that had been killed in alcohol or sodium fluoride showed the passage of more urea into the pure sugar solution than into Ringer's solution. The reason for this behavior is not known, unless it is that the constituents of the skin are not "fixed" so thoroughly by these chemicals as by formalin, and therefore the subsequent treatment with sugar causes more disorganization in the skin and more penetration than could occur in the latter case. This point is of chief interest in showing that the more viscous sugar solutions do not slow the rate of diffusion of urea more than does the Ringer's solution.

In Tables IX. to XII. are presented data typical of results obtained in a large number of experiments with dead skins. The skins were washed in the sugar or Ringer's solution respectively before and again after being killed in the solutions indicated. Penetration of the urea continued from three to three and a half hours.

Table IX.

3/19/26.

	Isotonic Sucrose + Ringer's Solution.		Ringer's Solution. Mg. Urea.
	Per Cent. Sucrose.	Mg. Urea.	
Killed in alcohol.			
"Normal" direction	50	15.66	10.68
	75	18.90	10.89
	100	21.12	10.50
"Turned"	50	15.99	9.51
	75	19.17	10.59
	100	19.71	11.16

Table X.

4/27/26.

	Isotonic Sucrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Normal" direction	1.74	2.07
	1.11	1.98
Killed in alcohol:		
"Normal" direction	19.83	12.63
"Turned"	17.49	11.58
Killed in NaF:		
"Normal" direction	5.82	5.04
"Turned"	6.45	4.95

Table XI.

5/12/26.

	Isotonic Dextrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Turned"	4.56	7.32
Killed in NaF:		
"Normal" direction	4.32	1.98
"Turned"	3.84	3.78
Killed in formalin:		
"Normal" direction	7.56	7.74
"Turned"	8.16	6.84
Killed in alcohol:		
"Normal" direction	24.48	18.48
"Turned"	26.82	21.78

Table XII.

5/18/26.

	Isotonic Dextrose Solution. Mg. Urea.	Ringer's Solution. Mg. Urea.
Living skins:		
"Normal" direction	3.54	5.16
Killed in NaF:		
"Normal" direction	12.74	10.08
"Turned"	5.58	3.84
Killed in formalin:		
"Normal" direction	3.48	3.00
"Turned"	3.90	5.46
Killed in alcohol:		
"Normal" direction	14.76	14.76
"Turned"	18.72	15.30

Discussion.

The experiments show that in the case of the shorter exposures the passage of urea through frog skin was checked by the presence of sugar. After about six hours, the time differing with different skins, the skins in a pure sugar solution were greatly injured, especially if the sugar was in contact with their inner surfaces. As a rule, skins in mixtures of sugar and salt solutions survived in proportion to the amount of salt present. In contrast to these conditions, skins bathed on the inner surface by Ringer's solution survived 24 hours and upward. Accordingly, when skins were exposed to the solutions for six hours or more, the skins in contact with the solutions containing the larger proportions of sugar were the more injured and permitted more urea to pass. Skins that had been killed in various ways showed more nearly the same degree of permeability in Ringer's and in sugar solutions.

As in all work on permeability of cells and tissues, it is necessary to distinguish carefully between the permeability caused by injury and that normal to tissues. It is not claimed that in these experiments the skins remained entirely uninjured during the time—three or four hours—that constituted the minimum exposure to the solutions used, which are quite abnormal for frog skin; but it is believed that the skins remained in a sufficiently healthy condition for this length of time to exercise a considerable degree of the selective permeability that is characteristic of living tissues. In spite of the fact that skins exposed to the sugar solutions even for the shorter periods would be expected to be proportionately more injured than those exposed to Ringer's solutions, more permeability to urea was shown in the latter condition.

Because of these results on urea it may be concluded, therefore, that the favorable effects of salts on the penetration of dissolved substances is not limited to the passage of other electrolytes as some authors have assumed, but applies to certain non-electrolytes as well. This is not surprising considering the universal presence and the known physiological importance of the electrolytes in living cells and tissues.

Summary.

1. Isotonic dextrose or sucrose solutions, as compared with Ringer's solution, check the passage of urea through frog skins if the exposure to the solutions is not so prolonged that the skins are injured.

2. When the skins are kept in contact with the solutions for such a length of time that those in the sugar solutions are injured or killed and those in Ringer's solution are still in a healthy state, then more urea may penetrate in the presence of the sugar solution.

3. Mixtures of isotonic sugar solution and Ringer's solution check the penetration of urea in proportion to the amount of sugar present when that amount reaches a concentration of 50 per cent. or more.

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